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The effects of fructooligosaccharide on the immune response, antioxidant capability and HSP70 and HSP90 expressions in blunt snout bream (*Megalobrama amblycephala* Yih) under high heat stress

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A B S T R A C T

This study evaluated the effects of fructooligosaccharide (FOS) on the immune response, antioxidant capability and HSP70 and HSP90 mRNA expressions in blunt snout bream (*Megalobrama amblycephala*) under high heat stress. A total of 360 fish were randomly distributed into three groups (each with four replicates) and fed with three levels of FOS (0, 0.4% and 0.8%) for 8 weeks. After the feeding trial, 20 fish per tank were exposed to high heat stress at 34 °C (ambient temperature + 8 °C). At 3 and 6 h after stress, both the plasma cortisol and glucose levels of fish fed with 0.4% FOS were significantly lower than those of fish fed with the control diets. This also held true for the lactate levels of fish fed with 0.4% FOS, except that a significant difference was only observed at 3 h. Plasma lysozyme, acid phosphatase (ACP) and alternative complement (ACH50) activities, as well as total protein, immunoglobulin M (IgM) and nitrogen monoxide (NO) contents, increased significantly, with maximum levels attained at 6 h for all parameters except for the ACP activity. Thereafter, these parameters all decreased significantly. In addition, fish fed with 0.4% or 0.8% FOS obtained significantly higher lysozyme, ACP and ACH50 activities as well as total protein, IgM and NO contents at 3 and/or 6 h after stress. The liver superoxide dismutase and catalase activities of fish fed with 0.4% FOS were both significantly higher than those of the control group before and after stress, while the opposite was true for the malondialdehyde content. After stress, the HSP70 and HSP90 expressions of fish fed with 0.4% FOS were both significantly higher than those of fish fed with the control diets at 3, 6 and 12 h (except the HSP90 at 12 h). Similar results were also observed in fish fed with 0.8% FOS except that the differences in HSP70 expression were only significant at 3 and 12 h. The results indicated that the supplementation of 0.4% FOS could increase the non-specific immunity, antioxidant capacity and HSP70 and HSP90 expressions of blunt snout bream and enhance its resistance to high heat stress.

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1. Introduction

Blunt snout bream (*Megalobrama amblycephala*), an herbivorous freshwater carp native to China, is one of the most important species cultured in China. In recent years, the aquaculture of blunt snout bream has expanded rapidly. However, this species is also threatened by many pathogens. At the same time, high temperature is the factor that most often limits the profitability and development of blunt snout bream and has resulted in great losses in its aquaculture. Given that temperature has a considerable influence on fish biochemistry, physiology and behaviour (Beitinger et al., 2000) and that elevated temperatures can have a negative influence on fish health by decreasing growth and increasing mortality (Dominguez et al., 2004), it is important to understand the effects of elevated water temperatures on this species' stress response and on various immune parameters. However, such data are relatively unavailable.

In the last decade, antibiotics have been frequently used to control bacterial infection and prevent fish mortality in aquaculture systems. However, the use of antibiotics has become limited because of negative effects, including the potential to develop antibiotic-resistant bacteria or leave antibiotic residue in seafood. Recently, probiotics or/and prebiotics have been suggested to improve the production and health of aquatic animals. Fructooligosaccharide (FOS), one of the most studied prebiotics, can improve host health by selectively stimulating the growth and/or activity of various beneficial bacteria. Studies in fish and shellfish have indicated that FOS could increase non-specific immunity and resistance to pathogens; protect them from oxidisation and rid them of free radicals (Ai et al., 2011; Soleimani et al., 2012; Zhang et al., 2010, 2013). However, little is known about the effects of FOS on the

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immunological responses and antioxidant capabilities of blunt snout bream under heat stress.

HSPs have received considerable attention in organisms because they modulate the cellular immune response and protect organisms from pathogenic stress. Members of both the HSP70 and HSP90 families are important in folding nascent polypeptides and renaturing heat damaged proteins (Morimoto, 1998). Previous studies have shown that exposing cells to stress conditions such as heat shock, oxidant injury, heavy metal pollution or bacteria activates heat shock genes, results in the synthesis of heat shock proteins (HSPs) (Leppa and Sistonen, 1997). Other studies have shown that the HSPs induced by high temperature can provide cross protection against other stressors (DuBeau et al., 1998; Li and Brawley, 2004; Renfro et al., 1993). But to the authors knowledge there is a little information on the heat shock response of this species under heat stress. Moreover, data on the effects of FOS on mRNA expression of HSP70 and HSP90 are scarce or unavailable. Therefore, we investigated the mRNA expression of HSP70 and HSP90 as an indication of their role in immune modulation in blunt snout bream.

Given the lack of information about how blunt snout bream respond to high temperatures in terms of their immunology and antioxidation, the objective of the present study was to evaluate the effects of FOS on immune parameters, hepatic antioxidant enzymes, and HSP70 and HSP90 expressions in blunt snout bream under high temperature stress. The data obtained will help clarify the mechanisms in the immune system of fish and the interaction among the host, FOS and environmental factors. The data can also provide us with further information about anti-stress mechanisms in blunt snout bream and help us select the appropriate additives to prevent or/and control diseases.

2. Materials and methods

2.1. Fructooligosaccharide (FOS) and experimental diets

The FOS used in this study was produced by Meiji Holdings Co., Ltd., Japan. The minimum level of sucrose combined with 1–3 fructoses in the product is 95%. Other components including glucose, fructose and sucrose account for less than 5%.

The formulation and proximate composition of the basal diet are presented in Table 1. Fish meal, soybean meal, cottonseed meal and rapeseed meal served as sources of protein. Equal portions of fish oil and soybean oil (1:1) were used as lipid sources. Wheat flour provided carbohydrates. Graded doses of FOS (0, 0.4 and 0.8%) were added to the basal diet at the expense of wheat flour to obtain the levels required. All of the diets were prepared in the laboratory. Dry ingredients were ground through a 60-mm mesh. The fine powder was carefully weighed and then mixed thoroughly with oil. An appropriate amount of water was later added to produce stiff dough. The dough was then pelleted (without injected steam) using a pellet mill with a 2-mm-diameter die. The experimental feed was air-dried at 33 °C overnight and stored in sealed plastic bags at 4 °C until use.

Table 1
Ingredients and proximate composition of the experimental diets (% of dry matter).

Ingredients	Control	0.4% FOS	0.8% FOS
Fish meal	8	8	8
Soybean meal	30	30	30
Cottonseed meal	15	15	15
Rapeseed meal	15	15	15
Soybean oil	2.2	2.2	2.2
Fish oil	2.2	2.2	2.2
Wheat bran	5	5	5
Wheat flour	19.6	19.2	18.8
$Ca(H_2PO_4)_2$	1.8	1.8	1.8
Premix[a]	1	1	1
Salt	0.2	0.2	0.2
(Fructooligosaccharide) FOS	0	0.4	0.8
Proximate composition (% air-dry basis)			
Moisture	11.44	11.44	11.44
Crude protein	32.71	32.71	32.71
Crude lipid	6.88	6.88	6.88
Energy (MJ kg^{-1})	15.08	15.08	15.08

[a] Premix supplied the following minerals (g kg^{-1}) and vitamins (IU or mg kg^{-1}): $CuSO_4 \cdot 5H_2O$, 2.0 g; $FeSO_4 \cdot 7H_2O$, 25 g; $ZnSO_4 \cdot 7H_2O$, 22 g; $MnSO_4 \cdot 4H_2O$, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; Vitamin A, 900,000 IU; Vitamin D, 200,000 IU; Vitamin E, 4500 mg; Vitamin K_3, 220 mg; Vitamin B_1, 320 mg; Vitamin B_2, 1090 mg; Vitamin B_5, 2000 mg; Vitamin B_6, 500 mg; Vitamin B_{12}, 1.6 mg; Vitamin C, 5000 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60,000 mg.

2.2. Fish and the feeding trial

Blunt snout bream were obtained from a commercial hatchery (Nanjing, China). Prior to the experiment, the fish were fed with a commercial diet (containing 32% protein and 6% lipids) during an acclimation period. After the acclimation, 360 fish (13.8 $\pm$ 0.04 g) were randomly stocked into each of 12 aquaria at 30 fish per tank. The fish were fed thrice daily at 7:00, 12:00 and 17:00 h to apparent satiation for a period of 8 weeks. Water temperature, pH and dissolved oxygen were monitored using a YSI 556 MPS multi-probe field meter (Geotech, USA). The water temperature was maintained at 25 $\pm$ 2 °C, and the pH fluctuated between 7.0 and 7.5. The DO and total ammonia nitrogen level were maintained above 5.0 mg l^{-1} and at less than 0.04 mg l^{-1}, respectively, during the feeding trial.

2.3. Heat stress experiment

After feeding for 8 weeks according to the method described in Liu et al. (2010), 20 fish of uniform size from each tank with water temperature of 26 °C were selected and transferred to a temperature-controlled aquarium at 34 °C for heat-shock treatment. In the experimental tank, the water temperature was maintained using an automatic heater, and DO > 5 mg/l. The fish were then anesthetised with MS-222 (ethyl 3-aminobenzoate methanesulfonate, Sigma-Aldrich, Germany) at a concentration of 100 mg/l. Blood samples were collected from four fish per tank before stress (0 h) and at 3, 6, 12, 24 and 48 h after heat stress. Blood samples were taken from the caudal vein using heparinised plastic syringes and then transferred immediately to heparinised capillary tubes. The blood was later centrifuged at 3000 *g* at 4 °C for 10 min. Plasma was collected and stored at −80 °C until analysis. In addition, the individual livers were quickly removed and stored at −70 °C for subsequent analysis.

2.4. Analysis

2.4.1. Proximate composition

The diets were analysed for proximate composition according to AOAC protocols (AOAC, 1990). Moisture was estimated by oven drying at 105 °C to a constant weight; crude protein was analysed by the micro-Kjeldahl method and multiplying by a factor of 6.25 after acid digestion using an Auto Kjeldahl System (1030-Auto-analyser, Tecator, Höganäs, Sweden); crude lipids were determined by solvent extraction with a Soxtec System HT (Soxtec System HT6, Tecator, Höganäs, Sweden); ash was determined by combustion at 550 °C for 4 h. Gross energy was measured by a Bomb Calorimeter (Parr 1281, Parr Instrument Company, Moline, IL, USA).

2.4.2. Stress response

Plasma cortisol concentration was estimated by a validated radioimmunoassay (RIA) method for fish as described by Winberg and Lepage (1998) using a commercial ^{125}Iodinecortisol RIA kit (ref. no. KD005-0049) produced by Beijing North Institute of Biological Technology (Beijing, China). Intra- and inter-assay coefficients of variation were 8.59% (n = 8) and 11.64% (n = 6), respectively. Plasma glucose levels were measured by the glucose oxidase method as described by Asadi et al. (2009). The plasma lactate concentration was determined by the

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